THE PASSIVE TRANSFERENCE OF NONSPECIFIC ANTIBODIES

PLATE 5

PETER K. OLITSKY AND BERNARD S. DENZER

From the Pathological Laboratory of the Mount Sinai Hospital, New York

In a previous communication[1] it was demonstrated that the injection of an antigen made from bacteria grown on serum medium could give rise to nonspecific antibodies. Such antigens, altho apparently washed free from the medium, stimulate the production not only of specific antibodies against the bacteria, but also of antibodies against the protein contained in the medium if the protein is heterologous; that is, derived from a species other than that of the animal used in immunization. These antibodies may react nonspecifically against any bacteria grown on such media. This factor may be the cause of the divergence in results in cross complement-fixation between the meningococcus and the gonococcus, or in that between dissimilar groups of streptococci, or between different organisms when the immune serum used in the tests has been prepared by injecting serum-grown bacteria into rabbits.

The object of this paper is to show that a similar phenomenon occurs in passive sensitization. Immune serum developed against repeated injections of bacteria grown on serum media, when inoculated into guinea-pigs, sensitizes the cells of this animal, not only to the bacteria, but also to the protein present in the medium on which the antigen has been grown.

Our attention was drawn to this fact while we were working with sensitized guinea-pig uteri by the Dale method.[2] We employed the technic as utilized by R. Weil in his study of pneumococcal sensitization.[3] In general the principles of this method are as follows:

A guinea-pig is sensitized by the subcutaneous injection of from 2 to 3 c.c. of the serum to be studied. After from 2 to 6 days the animal is killed and its uterus is removed; either horn is suspended in from 75 to 125 c.c. of Locke's solution. This solution is kept at a constant temperature of from 37 to 40 C. When the antigen is added to the fluid surrounding the uterus, there occurs a greater or lesser degree of contraction, the intensity of which is recorded on a drum covered with smoked paper. As a control, to test the

* Received for publication October 27, 1916. Work done under the tenure of George Blumenthal Jr. fellowships in pathology.

[1] Olitsky and Bernstein, Jour. Infect. Dis., 1916, 19, p. 253.

[2] Jour. Pharm. and Exper. Therap., 1913, 4, p. 167.

[3] Weil and Torrey, Jour. Exper. Med., 1916, 23, p. 1.

contractility of the uterine muscle, 0.0002 gm. of ergamine is added; this should give a contraction in a responsive or "live" uterus. After each addition of material to the Locke solution, the fluid is removed, the uterus washed, and fresh solution is added—all these procedures being conducted at a constant temperature.

In our earlier work, while experimenting with the passive transference of antibodies, we noted the following: A rabbit was immunized against bacteria grown on human-serum media. The immunization was effected by repeated injections of the antigen over a long period of time. Blood from this rabbit was injected into a guinea-pig, and after a few days the uterus was removed, suspended in the Dale apparatus, and tested. It was found then that not only did the uterus contract on the addition of antigen (see Fig. 1), but also on the addition of plain human serum (see Fig. 2). Thus we see that the antigen used in immunizing the rabbit had produced serum antibodies as well as specific bacterial antibodies.

A series of experiments was then undertaken with the object of investigating these phenomena.

A strain of B. typhosus was employed as antigen because the history of this culture was known: for years it has been transplanted and growing on plain agar. The organism in one instance was allowed to grow on plain agar; in the second, on human-serum agar.

Rabbit 1 received 7 intravenous injections over a period of 12 days, of saline suspensions of typhoid bacilli grown on plain agar. Between the 1st and 2nd injections there was an interval of 7 days, thereafter an injection was given daily. The 1st inoculation was made of dead typhoid bacilli (the growth on 1 agar slant); the subsequent inoculations, of live organisms, starting with 1 and ending with 6 loopfuls. Ten days after the last injection, the animal was bled and the titration of humoral antibodies showed: complement fixation, ++++ in 0.001 c.c. of serum; macroscopic agglutination, positive in 1:400 dilution. Altho the antibody content was low, it was sufficient for our purpose, since the Dale method detects the faintest possible trace of absorbed antibody. This, then, was the source of the "plain-agar typhoid immune serum."

Rabbit 2 was immunized in the very same manner, except that the antigen, altho consisting of the same strain of the typhoid bacillus, was made of the organisms grown on serum media. The first injection consisted of the same strain used in the case of Rabbit 1 grown for 3 generations on human-serum media. The 2nd to the 7th injections consisted of organisms of the 10th to the 15th generation, respectively. Care was always taken to exclude particles of the media from the suspension, which was washed in 0.9% salt solution 4 times. The supernatant fluid after each washing was tested for the presence of serum albumin and the suspension was used only when such test was negative. Ten days after the last injection the animal showed: complement fixation, +++ in 0.001 c.c. of its serum; macroscopic agglutination, positive in a dilution of 1:800. This was the source of the "serum-agar typhoid immune serum."

The experiments from this point were based on the principle that the

cells of the guinea-pig will absorb the antibodies from an immune serum and respond to the addition of a corresponding antigen. Accordingly, the "plain" and the "serum" typhoid immune sera (2.5 c.c.) were injected subcutaneously into guinea-pigs; 5 days later the animals were killed, and the uteri removed and suspended in Locke's solution, and tested.

In the case of the guinea-pig injected with the "plain" typhoid immune serum, it was determined that the uteri had absorbed typhoid antibodies (see Fig. 3a); there was no reaction to serum (see Fig. 3b and c).

In the case of the guinea-pig injected with the "serum" typhoid immune serum, it was determined that the uteri had absorbed typhoid antibodies (see Fig. 4) and at the same time antibodies against human serum (see Fig. 5).

DISCUSSION

It is possible that traces of the media may be carried over in the preparation of the antigens. We have kept this factor in mind and tried as much as possible to eliminate it. The possibility becomes still more remote when the degree of the nonspecific serum reaction is taken into account. It is difficult to conceive that minute traces of the media should so highly immunize the animal as to give the violent reactions noted in our experiments — reactions much more marked than those against the original antigens.

A great deal of evidence is rapidly accumulating showing the effect of media on the biologic variations of bacteria. Differences and changes in carbohydrate fermentation, in virulence, and in growth have been noted — some of these variations becoming permanent. The question arises whether one biotype transferred to a new environment will change its characteristics and become a new biotype developing along a pure line permanently. In our case, the typhoid bacilli growing on serum media absorb certain of the serum elements and become, so to speak, "serum" typhoid organisms. This may give rise to the nonspecific factors, for, theoretically, different bacteria may absorb the same chemical molecule from the serum of the same species. Wells and Osborne have recently demonstrated that anaphylactic specificity is dependent on the chemical constitution of the vegetable protein rather than on the species.

Several investigators have reported cross-fixations with different bacteria. In such instances the cross-fixation may be an evidence, not of the identity of the bacterial proteins, but of the identity of the proteins in the media. Whether the protein of the media is carried over into the antigen, or whether the bacteria absorb from the media certain common chemical molecules, we can not say.

The practical point of these experiments is that great care should be exercised in classifying bacteria by the ordinary method of testing against rabbit immune serum.

EXPLANATION OF PLATE

FIG. 1. This guinea-pig was sensitized to rabbit immune serum prepared against bacteria grown on serum media. The uterus shows a contraction on addition of the same antigen.

FIG. 2. The same uterus as in Fig. 1, showing a contraction even more marked on addition of human serum alone. The upper tracing shows the desensitization caused by the initial addition of the antigen, and the control contraction in response to ergamine.

FIG. 3. Uterus of a guinea-pig sensitized to rabbit immune serum prepared against "plain-agar" typhoid antigen (bacteria grown on plain agar).

a. Contraction in response to similar antigen.

b. No contraction in response to human serum.

c. Contraction in response to ergamine, showing uterus to be "alive."

FIG. 4. Guinea-pig uterus sensitized to rabbit immune serum developed against serum-grown typhoid antigen. Shows contraction in response to the same antigen.

FIG. 5. Same uterus as in Fig. 4, showing contraction in response to human serum.

Plate 5

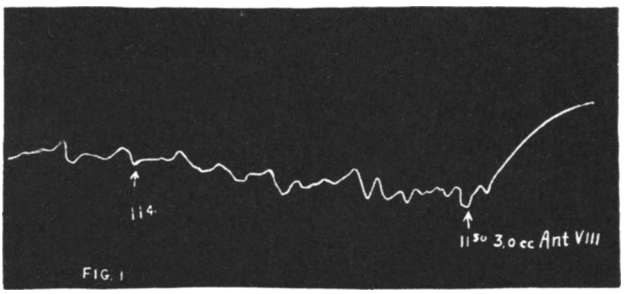

FIG. 1

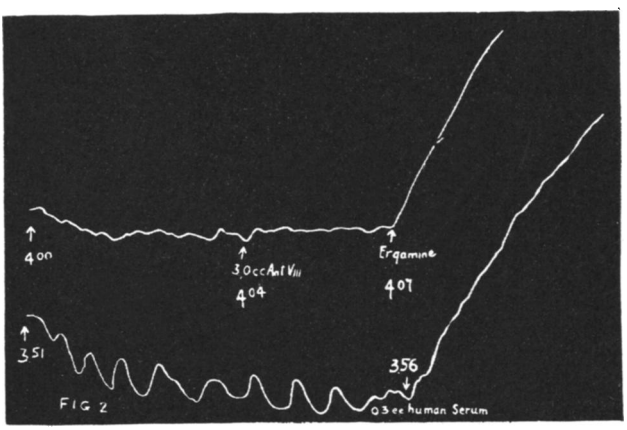

FIG 2